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JOURNAL ARTICLE

High Pressure Liquid Chromatography Techniques for the Isolation and Identification of Organics in Drinking Water Extracts

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Abstract

Some of the potential of high pressure liquid chromatography (HPLC) for sample cleanup of drinking water extracts for later analysis by GC/MS is described. Co-eluting compounds or background can be eliminated, thus improving mass spectra identification of GC peaks and enabling the identification of compounds not previously identified.

Introduction

Present methods for the analysis of organics in environmental extracts involve analysis by gas chromatography and mass spectrometry (GC/MS) usually by direct injection of an extract without additional cleanup. Problems encountered frequently include mixtures of compounds with overlapping retention times and a rising background that interferes with the interpretation of mass spectral data. High pressure liquid chromatography (HPLC) offers fast, sensitive techniques to selectively separate organics of interest from interfering background. Various methods have been reported using HPLC for isolating components from complex mixtures (1,2).

HPLC techniques provide a simple, efficient way of separating organics in drinking water extracts. Compounds can be separated and collected individually for analysis by probe-mass spectrometry, or they can be separated into various polarity groups and collected for analysis by GC/MS. The high background common to GC/MS analysis of many environmental samples can be reduced or eliminated by selective HPLC fractionation.

This paper describes some of the potential of HPLC for sample cleanup of extracts for later analysis by GC or GC/MS.

Equipment and Materials

Liquid Chromatograph and Columns—The high pressure liquid chromatograph used was a Waters Associates Model ALC/GPC 204 equipped with model 6000 pumps, a model 660 solvent programmer, and a 254 nm UV detector (Waters Associates, Milford, Massachusetts).

Columns—μ-Porasil (Waters Assoc.), 4 mm i.d. × 30 cm, flow rate of 2 ml/min. Gradient elution was started at 100% hexane and programmed to 30% chloroform/70% hexane in a linear manner over a period of 30 minutes.

μ-Bondapak-CN (Waters Assoc.), 4 mm i.d. × 30 cm, flow rate of 2 ml/min. Gradient elution was started at 100% hexane and programmed to 4% ethanol/96% hexane in a linear manner over a period of 20 minutes.

Gas Chromatograph and Mass Spectrometer—The GC/MS system used was a Finnigan 1015 mass spectrometer interfaced to either a Varian 1400 gas chromatograph or to a direct probe. GC columns used were 3% SE-30 on Supelcoport (2 mm i.d. × 180 cm) and 3% OV-17 on Supelcoport (2 mm i.d. × 210 cm). They were programmed from 60 to 240°C at 10°/min.

Reagents—Hexane, chloroform, and acetone were "Distilled-in-Glass" grade from Burdick & Jackson (Muskegon, Michigan). Ethanol was absolute, reagent quality from U.S. Industrial Chemicals (New York, New York). XAD-4 and XAD-8 are macro-reticular resins from Rohm and Haas Co. (Philadelphia, Pennsylvania).

Experimental

Athens Tap Water—Over a 9-day period in April 1976, 1000 liters of tap water were passed through a column (2.3 cm i.d.) containing 40 ml of a 50:50 mixture of XAD-4 and XAD-8 resin. The adsorbed organics were eluted from the resin with 120 ml of acetone followed by 480 ml of chloroform, and the mixed eluates evaporated to 1 ml using techniques described by Van Rossum and Webb (3).

The resin mixture had previously been exhaustively washed with acetone, methanol, and chloroform until a chromatogram of the chloroform washings showed no interferences by gas chromatography. No additional blanks were run.

The extract components were separated by HPLC by injecting 25 μl of the extract on a μ-Porasil column and eluting with a linear gradient of hexane to 30% chloroform/70% hexane (Figure 1). The major peaks were collected (1 to 2 ml volume) in 15-ml centrifuge tubes, evaporated with a gentle stream of dry nitrogen to between 10 to 100 μl volume, and analyzed by GC/MS or probe/MS.

Results and Discussion

The liquid chromatography μ-Porasil column with hexane and chloroform as eluting solvents separates compounds into several polarity ranges. Eluting with the solvent front are such

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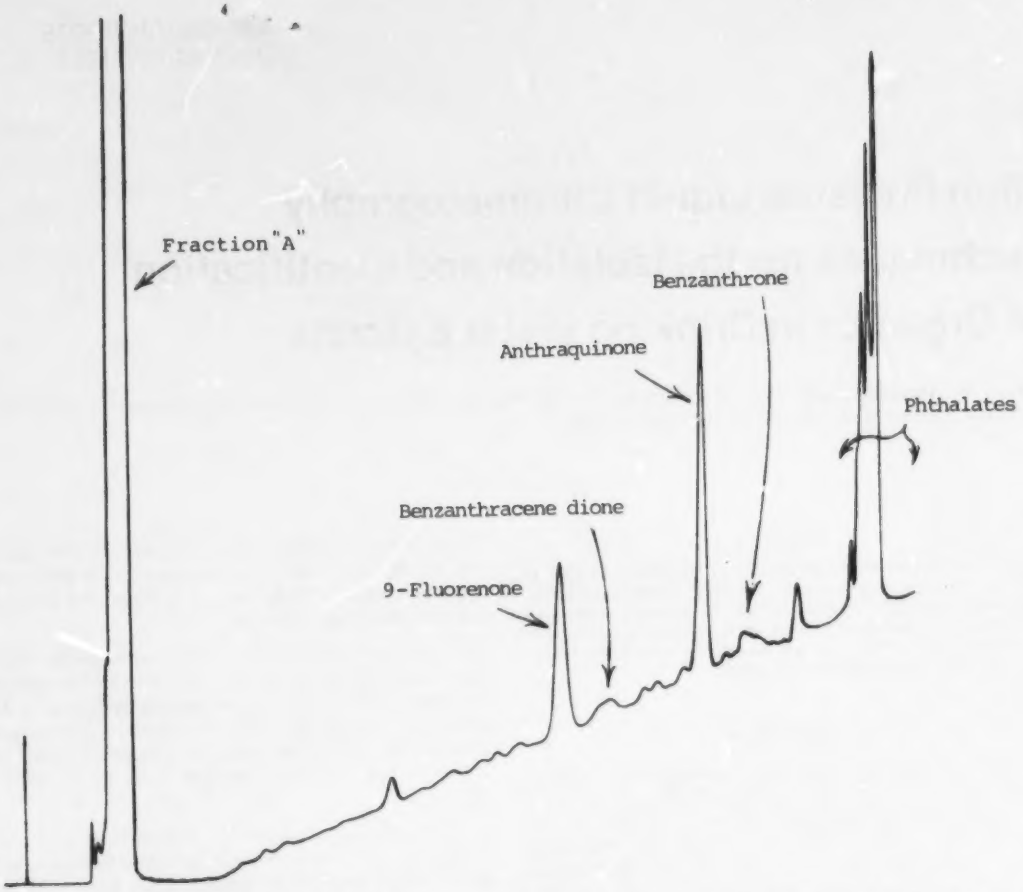

Figure 1. Liquid chromatography separation of Athens tap water extract. Column is μ-Porasil with solvent gradient of hexane to 30% chloroform/hexane. UV detector at 254 nm.

nonpolar types as polynuclear aromatic hydrocarbons, chlorinated hydrocarbons, and hydrocarbons (Fraction "A"); a middle range contains 9-fluorenone, benzanthracene-dione, anthraquinone, and benzanthrone. The phthalates (dimethyl to dioctyl), which are found in many environmental samples, come off in a plug near the end of this gradient program. If the percentage of chloroform in the gradient program is raised to 100%, an unresolved "blob" will elute. The components of this "blob" are believed to contribute to the continuously eluting background seen in gas chromatography analysis of many extract samples.

The Fraction "A" peak contains most of the compounds found by direct GC/MS analysis of the extract. Figure 2 shows Fraction "A" separated by GC/MS (SE-30 packed column) compared with the Athens tap water extract injected directly and run at the same conditions. Fraction "A" gives a much cleaner chromatogram, eliminating the rising background common in most extract samples.

The mass spectra of the peaks in the extract directly injected without cleanup were often so cluttered, even after background subtraction, that identification was often questionable when compared with known spectra. The mass spectra of each peak in the cleaned-up Fraction "A" were cleaner and less contaminated by background.

Computer matching of the peaks of the tap water extract and Fraction "A" were compared with reference spectra in the System Industries System/150 Library Search Program. Each match's reliability is indicated by the dissimilarity index (DI), a number between 0 and 1 that shows how well the unknown spectrum matches the found reference spectrum. The lower the number, the better the match. Limited experience has shown that a good match produces a dissimilarity index between 0 and 0.4.

Table I shows the DI values of the mass spectra on the two GC/MS runs. Some peaks in the tap water extract were so obscured by the background that they were not recognized as compounds by the computer search. LC Fraction "A" gave excellent matches (with low DI numbers) for each peak that had been identified in the sample before HPLC. In addition, some new compounds were identified: biphenyl, methyl biphenyl, methyl fluorene, and chrysene.

Quantitation—To obtain an idea of the quantitative range of these compounds in drinking water over a period of time, four tap water samples were taken during November-December 1976 using the XAD-4 and XAD-8 resin accumulator method. The eluted samples were chromatographed on the μ-Porasil LC column as previously described. Anthraquinone and 9-fluorenone were quantitated by LC on each extract by com-

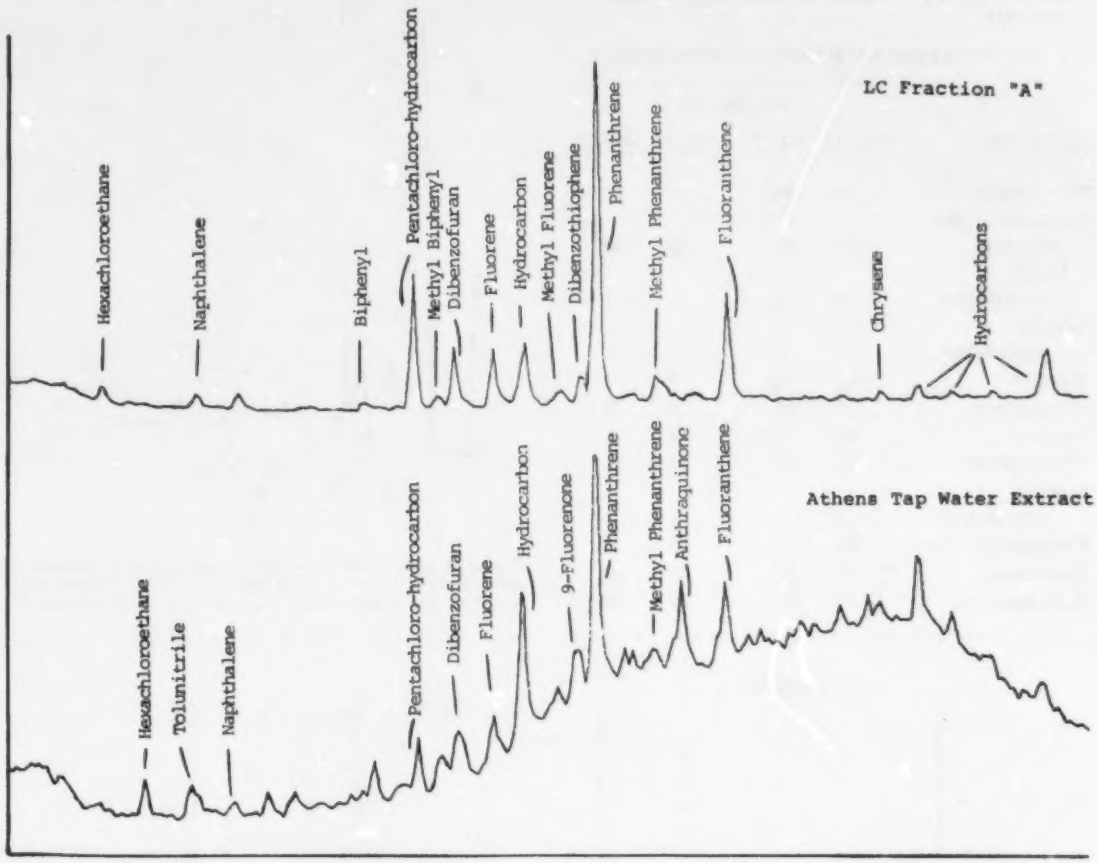

Figure 2. Reconstructed gas chromatogram of Athens tap water extract and LC Fraction "A" (see Figure 1). Column is 3% SE-30 on Supelcoport, programmed from 60°-240°C at 10°/min.

Table I. Dissimilarity Index for Two GC/MS Runs.

Compound	Athens, Georgia Extract	LC Fraction "A"
Hexachloroethane	0.199	0.175
Naphthalene	0.457	0.090
Biphenyl	—	0.452
Methyl biphenyl	—	0.329
Dibenzofuran	0.500	0.177
Fluorene	0.483	0.181
Methyl fluorene	—	0.546
Dibenzothiophene	—	0.569
9-Fluorenone	0.552	*
Phenanthrene	0.208	0.117
Methyl phenanthrene	0.736	0.381
Anthraquinone	0.560	*
Fluoranthene	0.364	0.142
Chrysene	—	0.519

*Not in Fraction "A."

paring with standards. Benzanthrone and benzanthracenedione, which were found in the earlier sample, were absent in these extracts. Fraction "A" of each sample was collected, chromatographed by GC, and quantitated by comparing with standards. Hexachlorocyclopentadiene was a new major compound found. Table II shows concentration values of the compounds found in the extracts, expressed as concentration in the original drinking water. These values are based on single runs and assume complete recovery. No study of recovery efficiencies of individual compounds onto and off the resin accumulator was done. Losses during evaporation of the LC fraction were not determined.

Other Column Techniques—The selection of a column for a liquid chromatographic separation requires consideration of the wide range of polarity present in most environmental samples. Polar bonded phases, such as the μ-Bondapak CN, are suitable for separation of molecules with polar functional groups.

The April 1976, Athens, Georgia, tap water extract was injected (25 μl) on a μ-Bondapak CN column (Figure 3) using a linear solvent gradient of hexane to 4% ethanol/hexane. Four fractions were collected, and each was analyzed by GC/MS.

Figure 4 shows the GC/MS chromatograms (OV-17 GC column) of the extract and two of the four collected LC fractions run all at the same conditions.

Table II. Organics in Athens, Georgia, Drinking Water Extracts, 1976.

(expressed as ng/liter of water—minimum values)

Compound	Sample run Nov. 16	Dec. 7	Dec. 16	Dec. 29
Naphthalene	16	10	6	10
Hexachlorocyclo-pentadiene	20	20	80	30
2-Methyl naphthalene	4	2	—	1
1-Methyl naphthalene	4	2	—	—
Biphenyl	5	2	1	2
Dibenzofuran	20	10	6	28
Fluorene	16	6	4	8
Phenanthrene	90	40	24	33
Methyl phenanthrene	2	1	1	1
Fluoranthene	24	7	5	6
Fluorenone	70	20	20	20
Anthraquinone	100	20	30	20

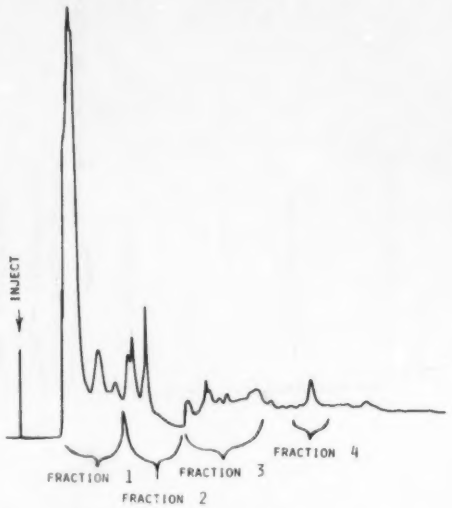

Figure 3. Liquid chromatography separation of Athens tap water extract. Column is μ-Bondapak CN with solvent gradient of hexane to 4% ethanol/hexane. UV detector at 254 nm.

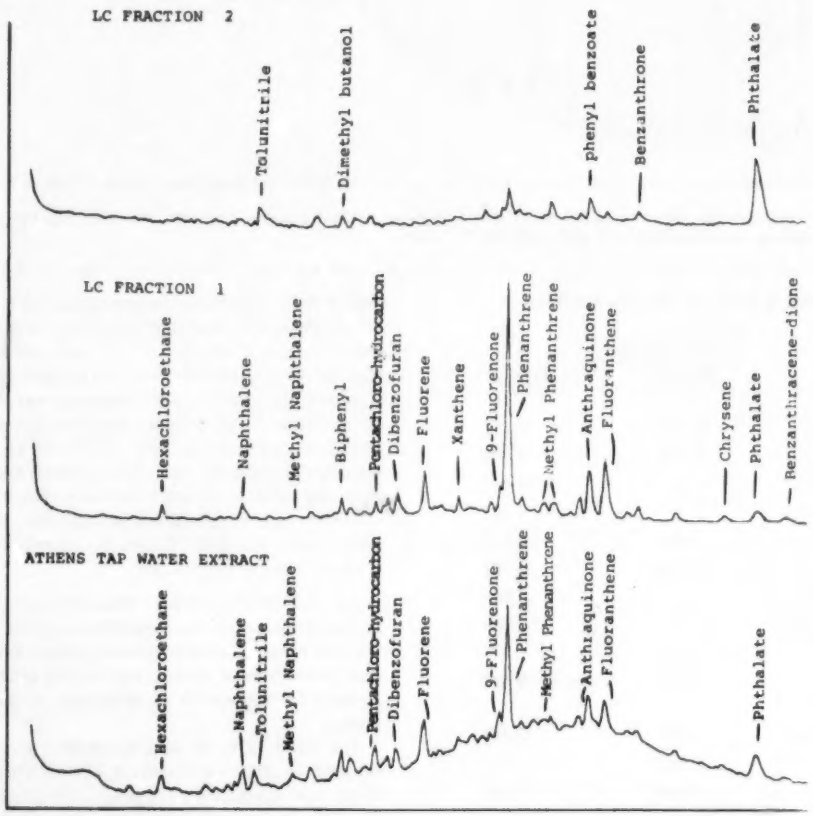

Figure 4. Reconstructed gas chromatogram of Athens tap water extract. Column is 3% OV-17 on Supelcoport, programmed from 60°-240° at 10°/min. Extract is lower chromatogram; LC Fraction 1 and LC Fraction 2 were collected from μ-Bondapak CN column (see Figure 3).

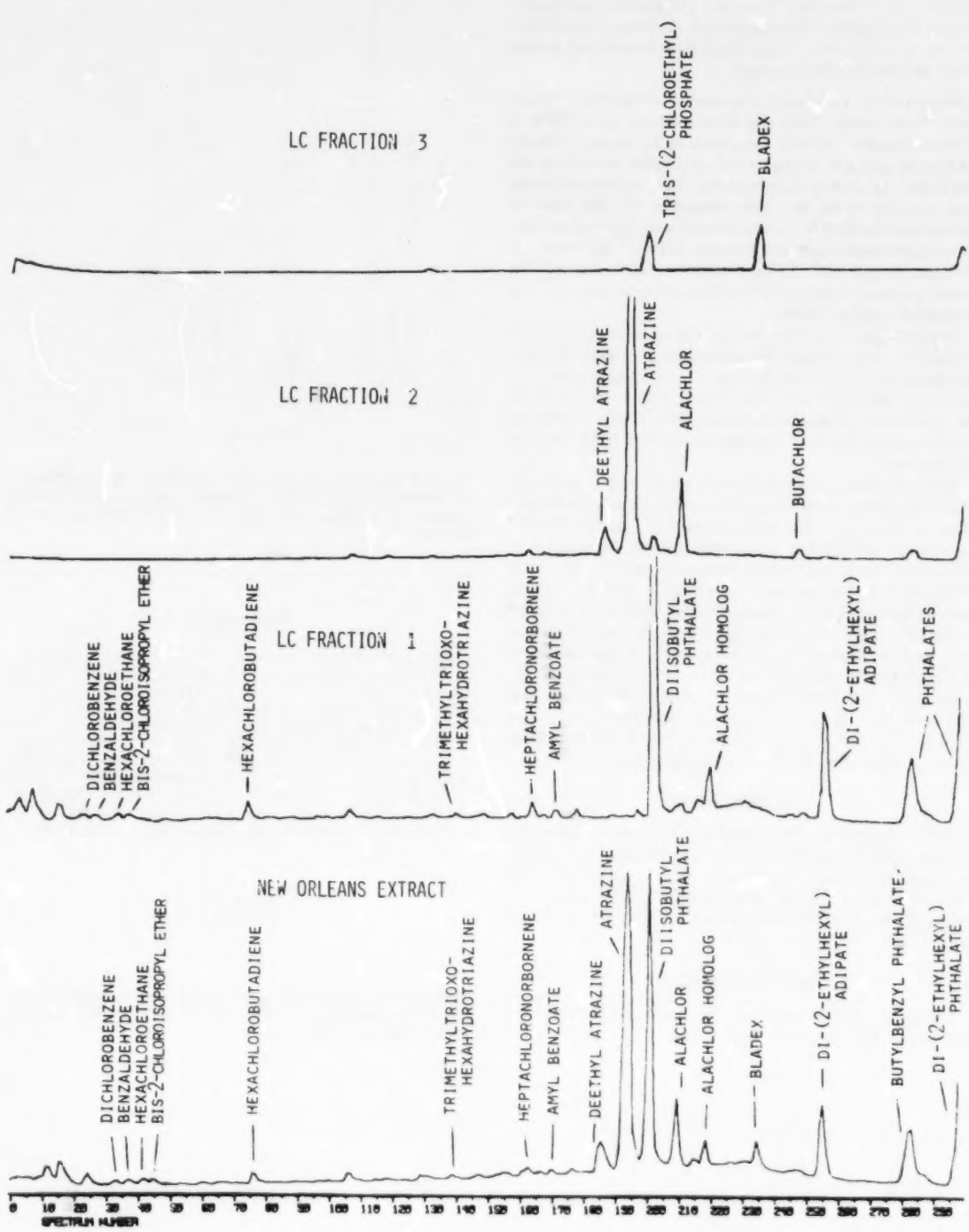

Figure 6. Reconstructed gas chromatogram of New Orleans tap water extract and three LC fractions collected from μ-Bondapak CN column (see Figure 5). GC column is 3% OV-17 on Supelcoport, programmed from 60°-240°C at 10°/min.

Fraction 1 contained most of the compounds with no high background interference. Fraction 2 contained the more polar tolunitrile, dimethyl butanol, phenyl benzoate, and benzanthrone. Fractions 3 and 4 contained trace amounts of mesityl oxide and methyl phenyl acetate.

Elimination of Co-eluting Compounds—The New Orleans Area Water Supply Study was undertaken the fall of 1974. A "Mega Sample" extract was obtained by passing 300,000 gallons of tap water through a carbon column; the carbon was dried and extracted with chloroform. This chloroform extract was analyzed by GC/MS and reported in 1975 (4). Over 80 compounds (in the 0.01 to 3 μg/l range) were reported—all different from those found in the Athens, Georgia, tap water. LC techniques were used on this sample to determine whether better separation and identification could be done on this extremely complex sample.

Figure 5 shows the New Orleans tap water extract injected (25 μl) on a μ-Bondapak-CN column using a solvent gradient of hexane to 4% ethanol/hexane. Three fractions were collected and analyzed by GC/MS. Figure 6 (shown at left) shows the reconstructed gas chromatogram (OV-17 GC column) of the extract and the three collected fractions (all run at the same conditions).

It is interesting to note in Figure 6 that the very large peak of diisobutyl phthalate in the extract could be completely isolated into LC Fraction 1. The peaks isolated in Fractions 2 and 3 gave mass spectra not contaminated with background or phthalate. *Tris*-(2-chloroethyl) phosphate, mol wt 284, found in Fraction 3, had not been identified in the New Orleans water prior to this work. This is the same compound identified in the 1975 report (4) as "unknown dichlorinated compound, mol wt 249." At that time the background and diisobutyl phthalate had so masked this spectrum, true identification was impossible. This is an example of using liquid chromatography techniques to improve mass spectral identification. The high background common to many environmental samples can be eliminated by selective HPLC fractionation.

Conclusions

Liquid chromatography can be used to improve the quality of mass spectra from GC/MS runs of environmental extracts by eliminating co-eluting compounds or background, thus improving mass spectra identification of GC peaks and enabling the identification of compounds not previously identified.

Acknowledgment

Ronald G. Webb is gratefully acknowledged for providing the Athens, Georgia, tap water extracts.

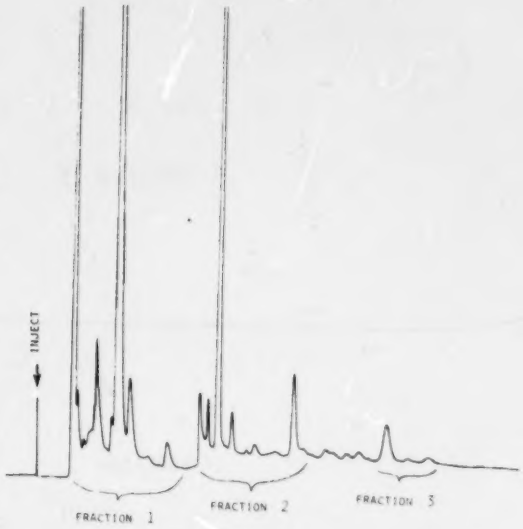

Figure 5. Liquid chromatography separation of New Orleans tap water extract. Column is μ-Bondapak CN with solvent gradient of hexane to 4% ethanol/hexane. UV detector at 254 nm.

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